

U.S.S.N. 09/991,152

Filed: November 16, 2001

AMENDMENT AND RESPONSE TO OFFICE ACTION

In the Claims

1. (currently amended) A genetically engineered organism selected from the group consisting of bacteria and plants producing polyhydroxyalkanoate (PHA), the improvement comprising providing the organism with a transgene encoding an enzyme ~~selected from the group consisting of medium chain length PHA synthases, acyl-CoA synthetases, acyl CoA transferases and~~ having the catalytic activity of 3-hydroxyacyl-ACP thioesterases, wherein at least one of the enzymes is encoded by a transgene, so that medium chain length PHA accumulates through the fatty acid biosynthesis pathway.
2. (original) The organism of claim 1 further comprising one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase.
3. (original) The organism of claim 2 wherein the acyl-CoA synthetase is 3-hydroxyacyl-CoA synthetase.
4. (currently amended) The organism of claim 2 ~~wherein the~~ comprising a transgene alkK encoding an acyl-CoA synthetase is alkK.
5. (currently amended) The organism of claim 2 ~~or 3 further~~ expressing a heterologous 3-hydroxyacyl-CoA synthetase activity.
6. (currently amended) The organism of claim 1 ~~further~~ expressing a heterologous 3-hydroxyacyl-CoA synthetase activity.
7. (previously presented) The organism of claim 1 wherein the enzyme is modified to enhance expression in the genetically engineered organism.

U.S.S.N. 09/991,152

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8. (previously presented) The organism of claim 1 expressing an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.

9. (original) The organism of claim 8 further expressing selectable marker genes, wherein the organism is a whole plant.

10. (previously presented) The organism of claim 1 expressing an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA synthetase, wherein the organism is a bacteria.

11. (original) The organism of claim 8 wherein expression of the transgene is targeted to a tissue or organelle selected from the group consisting of seeds, leaf, plastids, and peroxisomes.

12. (original) The organism of claim 10 wherein the bacteria is *E. coli* and PHA accumulates within the bacteria.

13. (currently amended) A method of engineering a PHA biosynthetic pathway in a transgenic organism selected from the group consisting of bacteria and plants which produce polyhydroxyalkanoate (PHA), the improvement comprising providing the organism with a construct comprising a transgene encoding an enzyme ~~selected from the group consisting of medium chain length PHA synthases, acyl-CoA synthetases, acyl-CoA transferases and~~ having the catalytic activity of 3-hydroxyacyl-ACP thioesterases, so that medium chain length PHA accumulates through the fatty acid biosynthesis pathway.

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3

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077832/00140

U.S.S.N. 09/991,152

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AMENDMENT AND RESPONSE TO OFFICE ACTION

14. (original) The method of claim 13 wherein the construct further comprises one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase.

15. (previously presented) The method of claim 14 wherein the construct comprises a transgene encoding a 3-hydroxy acyl-CoA synthetase.

16. (original) The method of claim 15 wherein the construct further comprises a transgene encoding a PHA synthase.

17. (original) The method of claim 16 wherein the organism is a plant.

18. (original) The method of claim 16 wherein the construct expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.

19. (original) The method of claim 16 wherein the construct expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA synthetase, wherein the organism is a bacteria.

20. (currently amended) A method of making medium chain length PHA comprising growing a transgenic organism selected from the group consisting of bacteria and plants, the organism producing polyhydroxyalkanoate (PHA) and expressing at least one enzyme selected from the group consisting of medium chain length PHA synthases, acyl CoA synthetases, acyl

U.S.S.N. 09/991,152

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~~CoA transferases, and a transgene encoding an enzyme having the catalytic activity of 3-hydroxyacyl-ACP thioesterases, with substrates for fatty acid biosynthesis.~~

21. (previously presented) The method of claim 20 wherein the organism further comprises one or more transgenes encoding enzymes having the catalytic activity of acyl-CoA synthetase or acyl CoA transferase.

22. (original) The method of claim 21 wherein the acyl-CoA synthetase is 3-hydroxyacyl-CoA synthetase.

23. (currently amended) The method of claim 21 wherein the organism further express a PHA synthase ~~transgene~~.

24. (currently amended) The method of claim 22 wherein the organism further express a PHA synthase ~~transgene~~.

25. (withdrawn) The method of claim 24 wherein the organism expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, medium chain length PHA synthase, and medium chain length 3-hydroxy fatty acid acyl CoA synthase, wherein the organism is a plant cell, plant tissue, or whole plant.

26. (original) The method of claim 24 wherein the organism expresses an enzyme selected from the group consisting of 3-hydroxyacyl-ACP thioesterase, a PHA synthase that incorporates medium chain length hydroxy acids, and medium chain length 3-hydroxy fatty acid acyl CoA synthetase, wherein the organism is a bacteria.

27-28. (cancelled)

29. (original) The organism of claim 10 wherein the bacteria is *E. coli*, and

U.S.S.N. 09/991,152

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wherein 3-hydroxy acids are secreted into the culture medium.

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6

MBX 041
077832/00140